

495  
↓

human eNOS:	AAKGTGITRKKT	-FKEVANAVKISASL	MGTVMAKRVKA (SEQ ID NO: 2)
bovine eNOS:	ATKGAGITRKKT	-FKEVANAVKISASL	MGTLMKRVKA (SEQ ID NO: 3)
human iNOS:	DEK-RRPKRREI	PLKVLVKAVLFACML	MRKTMASRVRV (SEQ ID NO: 4)
rat iNOS:	DEK-LRPRRREI	RPTVLVKAVFFASVL	MRKVMASRVRA (SEQ ID NO: 5)
mouse iNOS:	NEK-LRPRRREI	RFRVLVKVFFASML	MRKVMASRVRA (SEQ ID NO: 6)
human nNOS:	GTNGTPTKRRAI	GFKKLAEAVKFSAKL	MGQAMAKRVKA (SEQ ID NO: 7)
rat nNOS:	GTNGTPTKRRAI	GFKKLAEAVKFSAKL	MGQAMAKRVKA (SEQ ID NO: 8)

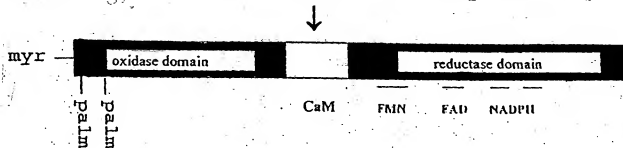


FIGURE 1

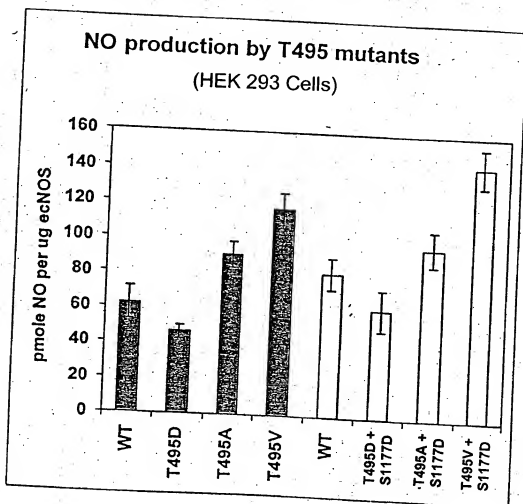
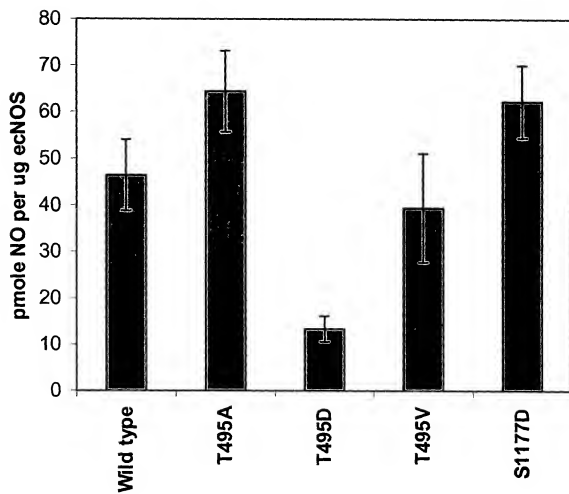


FIGURE 2

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FIGURE 3

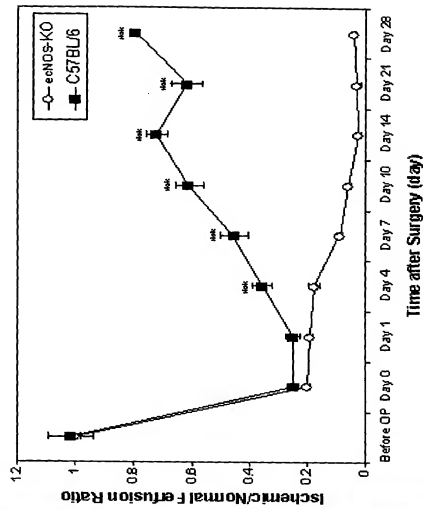
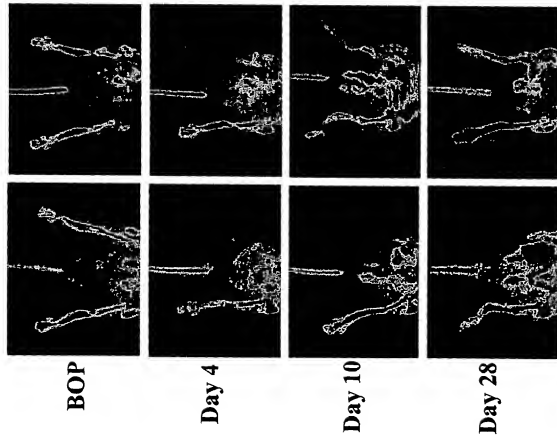


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FIGURE 4: Wt/ecNOS-KO LDPI Flow

ecNOS-KO

C57Bl/6



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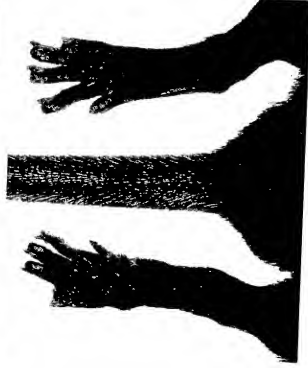
FIGURE 5: WT/ecNOS-KO gross pathological changes

Day 0



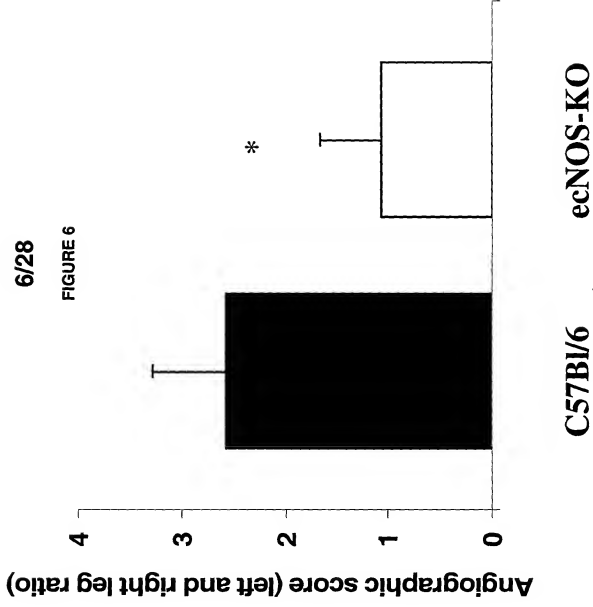
ecNOS-KO

Day 4



C57BL/6





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Figure 7: Different Surgical Procedures for CLI

Normal  
Anatomy



Surgical  
Procedure

Whole Fem. A. & V.  
Resection



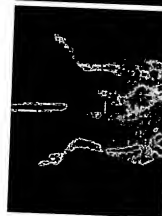
Whole Fem. A.  
Resection



Segmental Fem. A.  
Resection



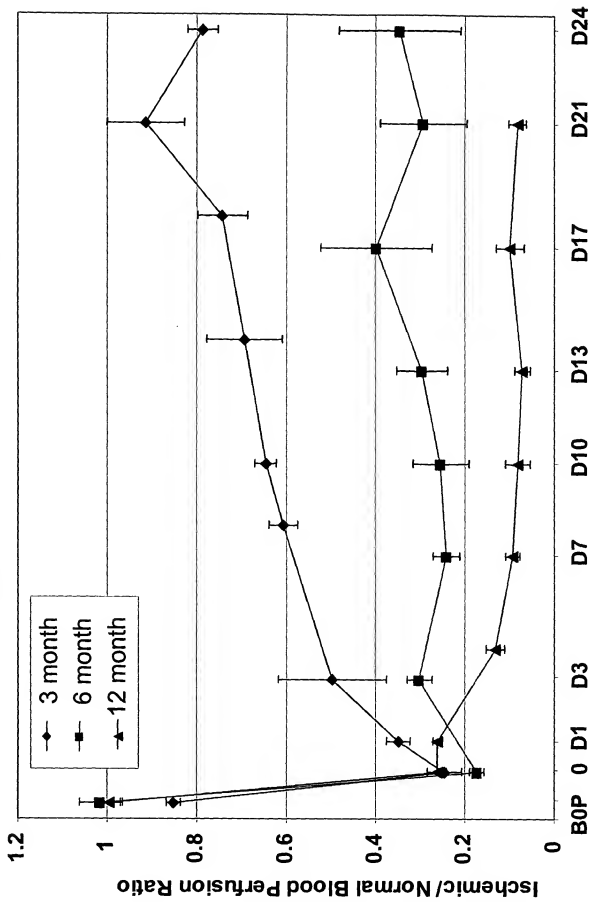
Gross  
Pathology



LDPI Flow



FIGURE 8: Effect of age on spontaneous blood flow recovery

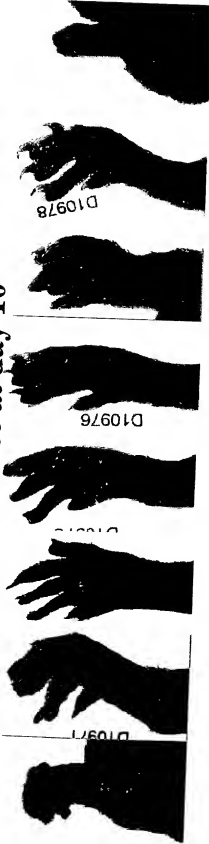




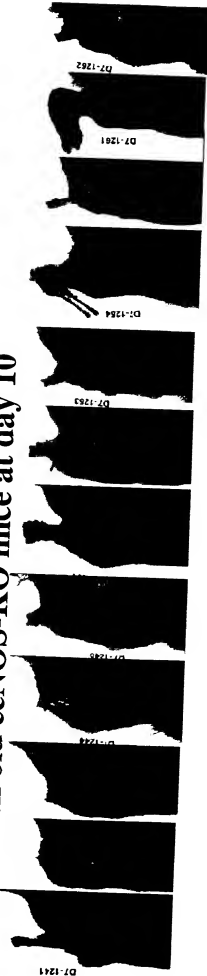
3 month old ecNOS-KO mice at day 10



6 month old ecNOS-KO mice at day 10



11-12 month old ecNOS-KO mice at day 10

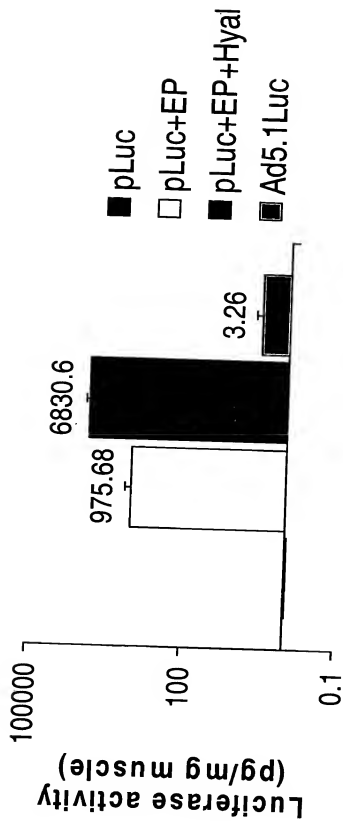


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FIGURE 9: Effect of age on ischemic damage.

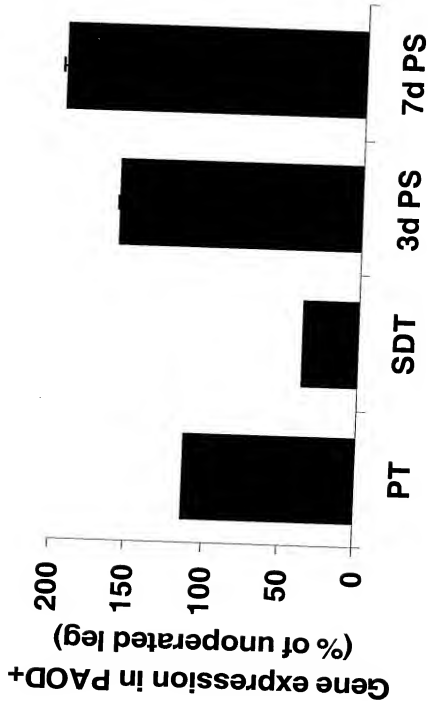
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FIGURE 10



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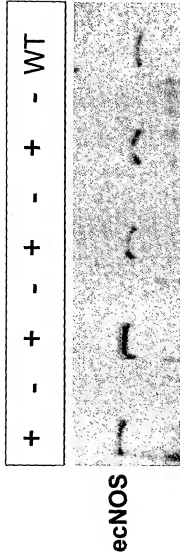
FIGURE 11



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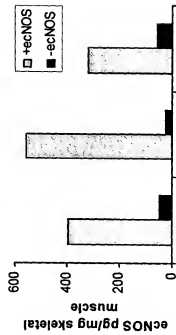
FIGURE 12: ecNOS expression in ecNOS-KO mice following gene therapy

### Western blot for ecNOS



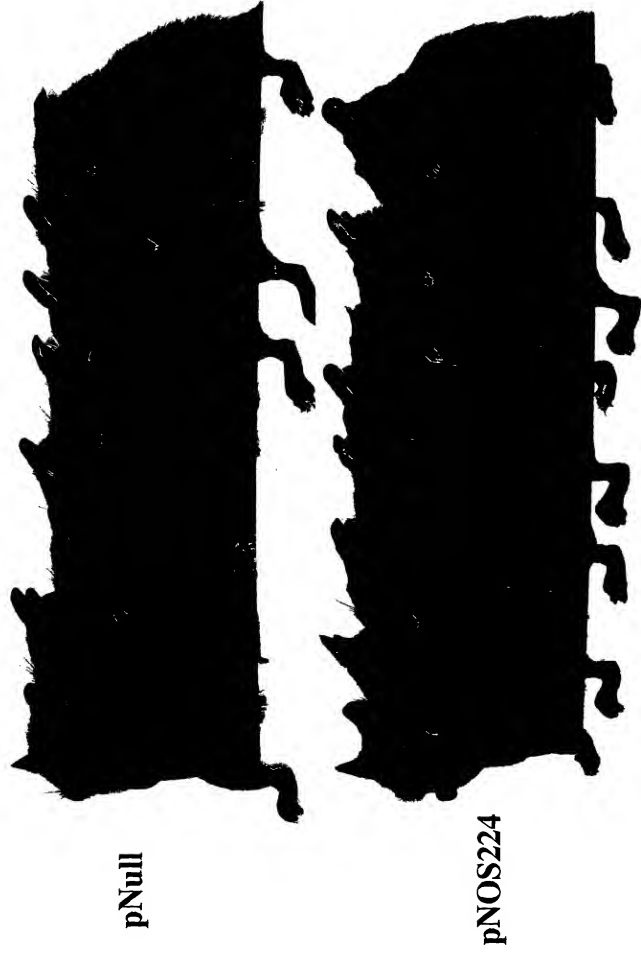
### ecNOS ELISA

(ecNOS-KO mice, pNOS224+EP)



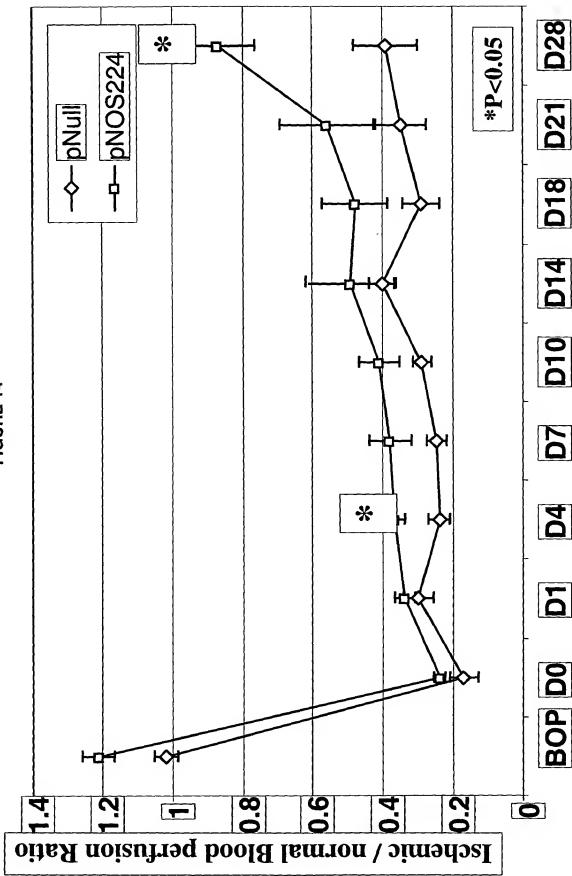
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**FIGURE 13:** Limb salvage after pNOS224 delivery in 6 month old ecNOS mice.

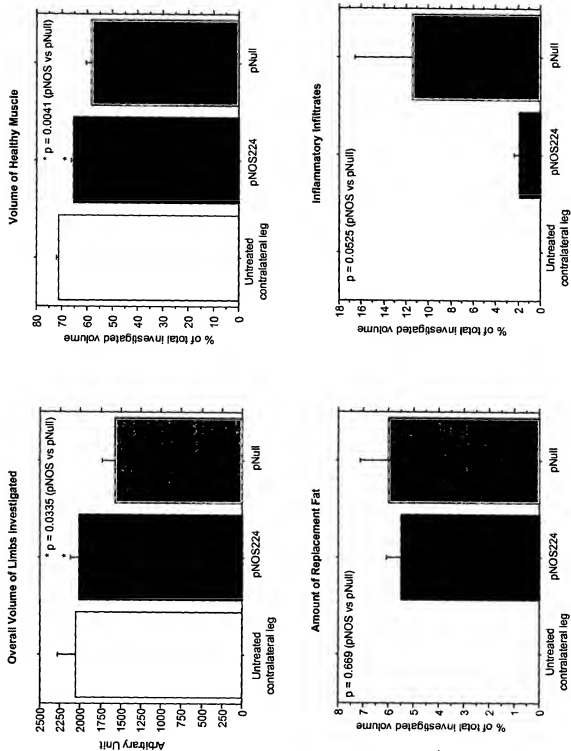


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FIGURE 14

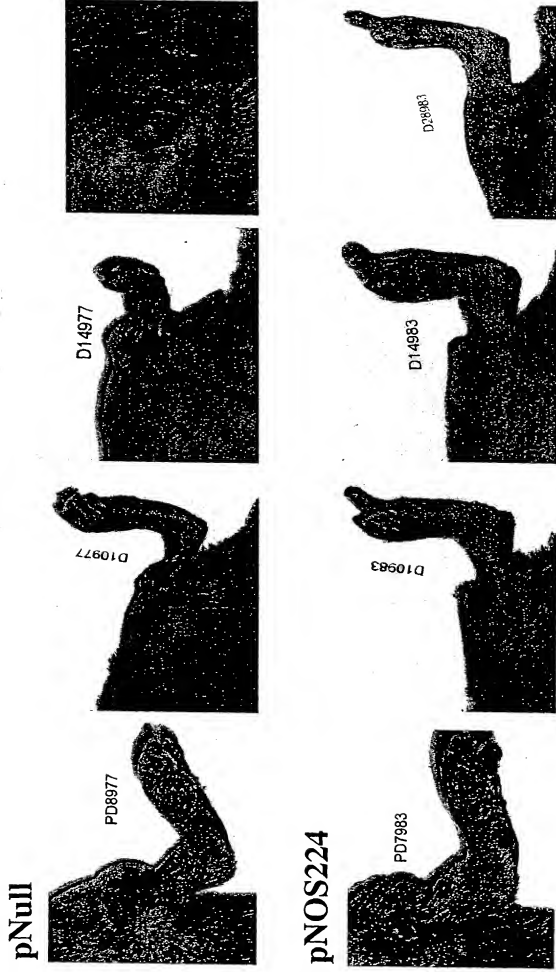


**FIGURE 15:** Histomorphometric analysis of pNOS224 treated hindlimbs vs. empty vector treatment



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FIGURE 16: Healing of skin ulcers in pNOS224 treated mice





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FIGURE 17: Limb salvage in 11-12 month old mice

pNull

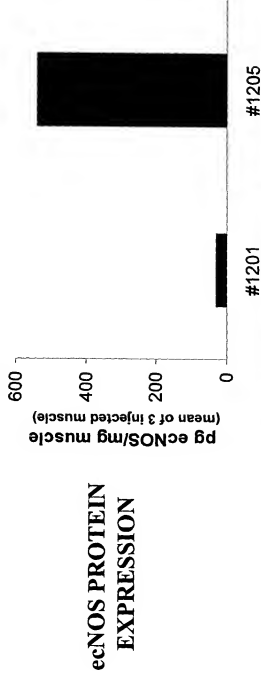


pNOS224



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FIGURE 18: ecNOS protein expression and therapeutic effect of gene delivery



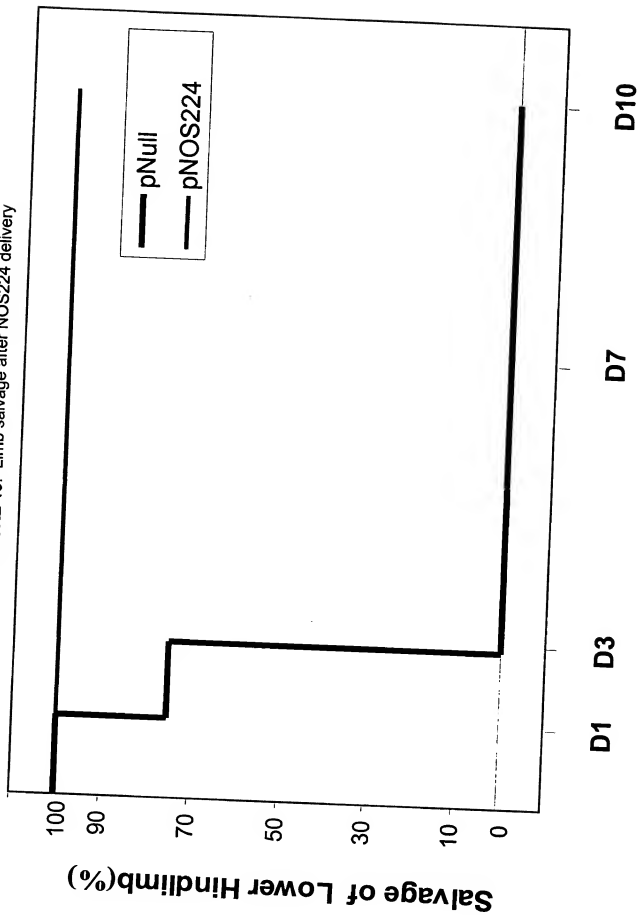
**BLOOD FLOW  
(LDPI)**



**LIMB NECROSIS**

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FIGURE 19: Limb salvage after NOS224 delivery



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FIGURE 20: Effect of NOS224 treatment on LDPI measured flow

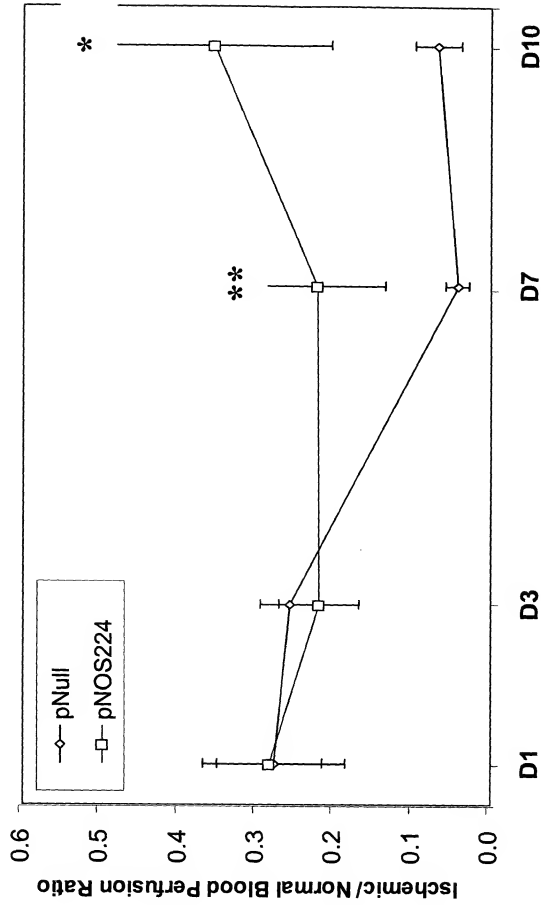


FIGURE 21: ecNOS protein expression in the adductor muscle of ecNOS-KO mice

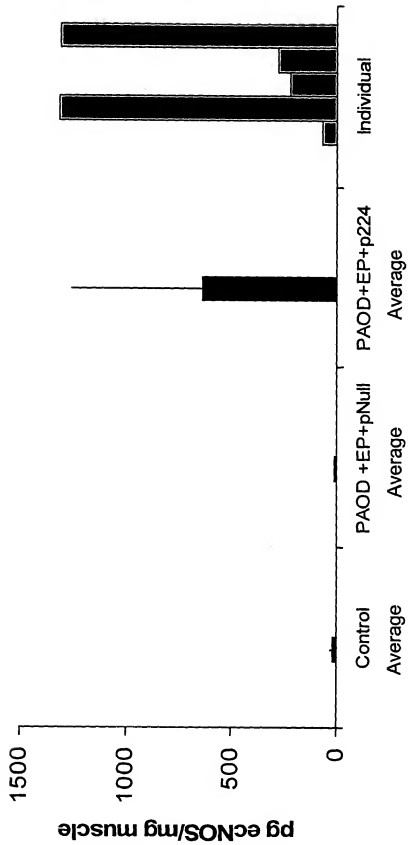
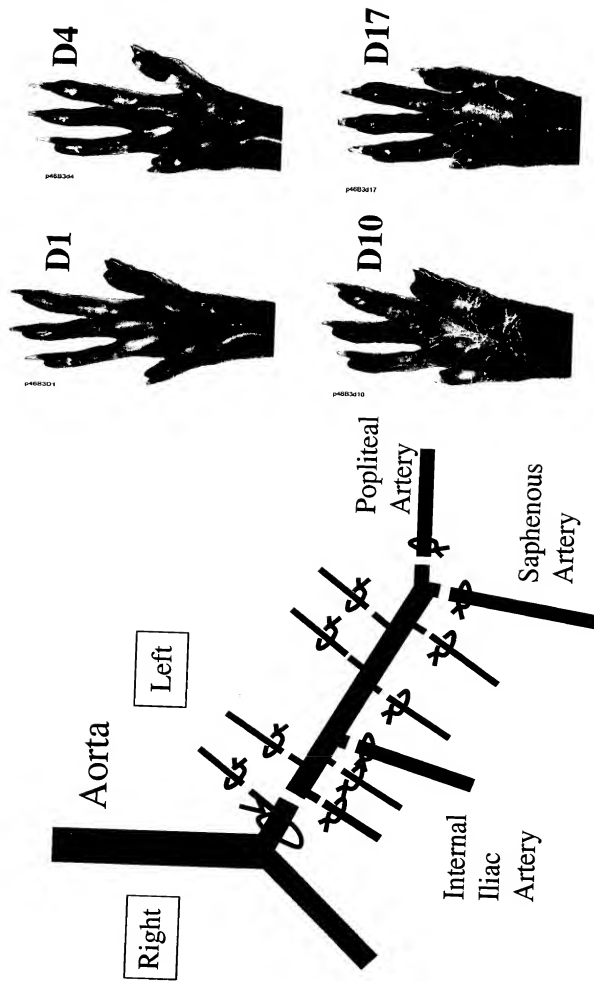


FIGURE 22: CLI Rat Model



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FIGURE 23: Angiography in Rat

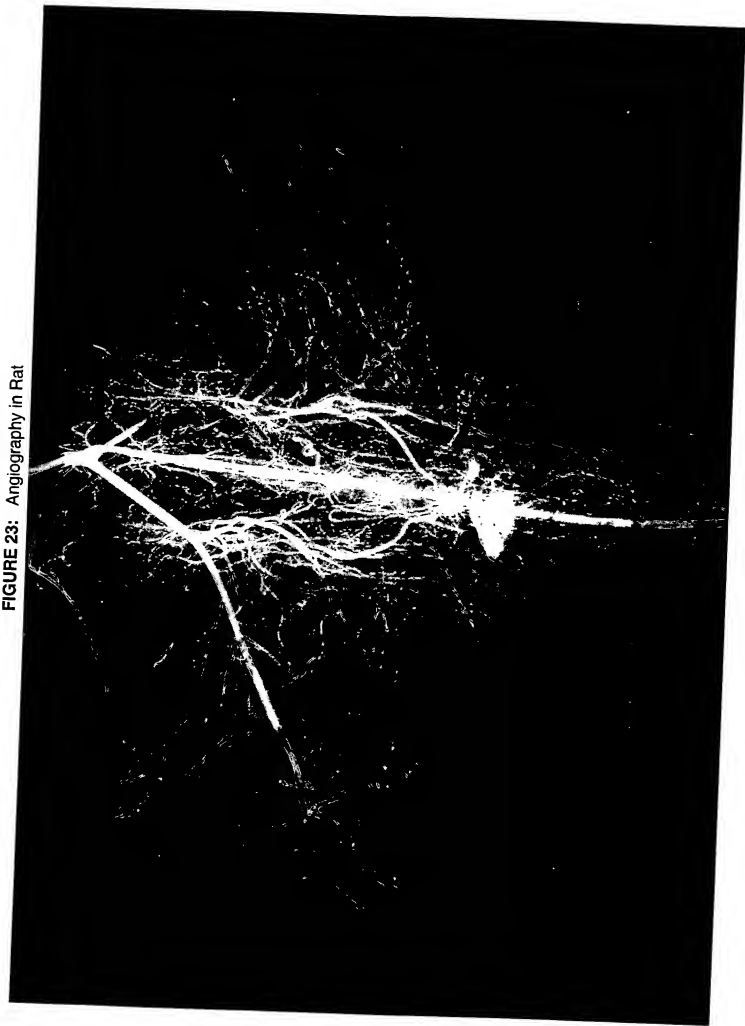


FIGURE 24: Angiographic Score



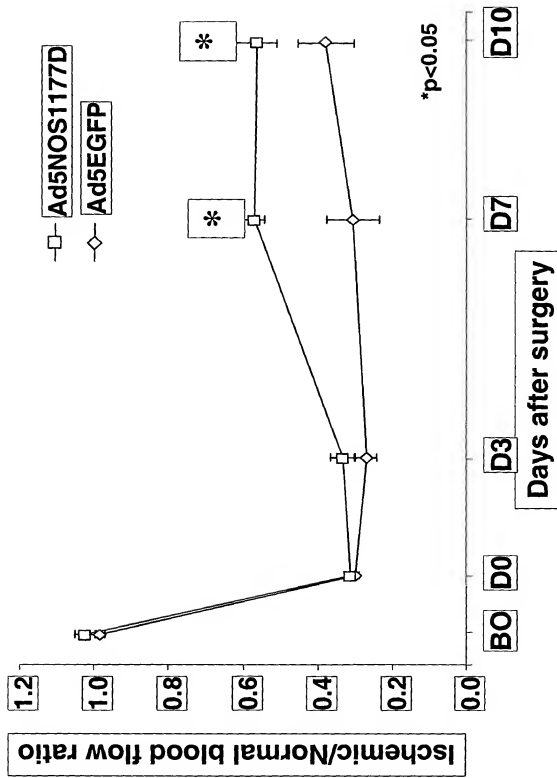
To quantitate arteriogenesis, three straight lines started from internal 1/4, middle, and external 1/4 of the femur were drawn at the medial thigh area of both normal and ischemic limb, and total number of arteries crossing these lines were calculated by **two separate investigators blinded to the treatment**.

To minimize variations, angiographic score was expressed as the ratio of total artery number of left to right hindlimb.








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FIGURE 7: Blood flow recovery after Ad5NOS delivery



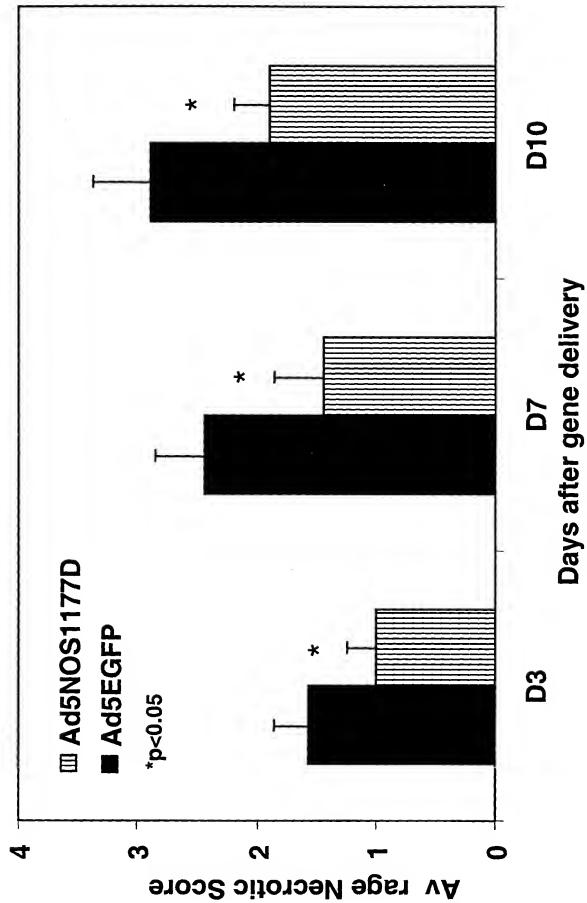
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FIGURE 26: NECROTIC SCORE

STAGE			GROSS PATHOLOGY	SCORE
Stage I			Normal situation or nail necrosis	0
Stage II			Toe(s) necrosis or discoloration	1
Stage III			Toe(s) loss or paw necrosis	2
Stage IV			Loss of all toes or partial paw	3
Stage V			Loss of more than half paw	4

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FIGURE 27



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FIGURE 28

